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Influence of Different Carbohydrate Sources and Fermentation Duration on the Physical and Chemical Quality of Trembesi Leaf Silage

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The availability of forage throughout the year is crucial, and therefore, efforts are needed to preserve forage through silage. Silage is a forage preservation technology that utilizes microbial activity to enhance the quality of the forage as animal feed. Trembesi is a perennial plant with a high protein content and abundant availability. However, high-protein feed materials can hinder the ensilage process. The aim of this study is to determine the physical and chemical quality of Trembesi leaf silage with the addition of different carbohydrate sources and ensiling durations, evaluated through *in vitro* analysis. The treatments applied in this study involved using various carbohydrate sources and different ensiling durations in the production of Trembesi leaf silage. A completely randomized design with a factorial pattern was used, where the first factor was the use of five different carbohydrate sources (bran, broken rice, molasses, cassava flour, and corn flour), and the second factor was the different ensiling durations (0, 7, 14, and 21 days). The parameters observed included physical quality (color, aroma, and texture), chemical quality (DM, OM, CP, CF, EE, ash, NFE, TDN, and lactic acid production). The conclusion of this study is that the addition of molasses as a carbohydrate source to Trembesi leaf silage ensiled for 21 days resulted in the best physical and chemical quality.

Keywords: Animal feed, forage, ruminant farming, carbohydrate sources, chemical quality, fermentation duration, lactic acid bacteria, *Samanea saman*

INTRODUCTION

Feed is one of the key factors in livestock farming, alongside breeding and management. The availability of feed in sufficient quality, quantity, and continuity greatly affects the success of livestock farming. One of the challenges in ruminant farming is the availability of forage. The availability of forage with good quality, high quantity, and sustainable supply is highly fluctuating due to Indonesia's climate conditions. To ensure continuous feed availability, steps must be taken to find alternative feed sources, including preservation techniques and alternative forage sources. One widely developed forage preservation method is silage (Wijayanti et al., 2022).

Trembesi is an evergreen perennial plant and has been recommended by the Indonesian government for development due to its suitability for reforestation, as it absorbs CO_2 and thus is abundant in Indonesia (Sariri & Kustantinah, 2021). Trembesi is a fast-growing plant, which accelerates its growth into a large tree. To control this rapid growth, pruning of the leaves is conducted. The pruned leaves can be used as livestock feed. Livestock are fond of Trembesi leaves because they contain 20.83% protein. However, the leaves also contain anti-nutritional substances, such as saponins, which can cause bloating and reduce appetite in animals. Several studies have been conducted to reduce the anti-nutritional content, particularly saponins and tannins, through fermentation. Silage is an application of forage preservation technology that utilizes the fermentation process. The success of silage depends on the ensiling process (Raguati et al., 2022).

The process of making silage, or ensiling, is a controlled fermentation process that promotes the growth of lactic acid-producing bacteria. The formation of silage occurs due to the

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conversion of water-soluble carbohydrates (WSC) by bacteria into lactic acid, which causes the pH to drop to around 4. This low pH is a result of lactic acid production. Therefore, the use of superior lactic acid bacteria (LAB) as a starter in the silage process is crucial (Jasin, 2014).

Trembesi leaves, despite their high protein content, do not benefit the ensiling process or the growth of lactic acid bacteria (LAB). High protein content in silage materials is undesirable because it does not serve as a substrate for lactic acid bacteria. Additionally, high protein levels can create a buffer capacity, which is the ability to maintain environmental pH balance (Handajani, 2014). This buffer capacity disrupts the fermentation process. The success of fermentation, particularly in silage, depends on an acidic environment or a low pH (a pH level of 4 or below). To maintain this acidic condition, additives such as microbes that can withstand low pH conditions and an abundant supply of water-soluble carbohydrates are needed. When ruminants are slaughtered, rumen contents are produced as waste, which can contribute to environmental pollution. Rumen contains millions of microbes, including lactic acid bacteria. LAB from rumen waste can be isolated and selected based on their resistance to low pH. LAB that can survive in low pH conditions are expected to thrive in silage, where a pH drop occurs due to the conversion of carbohydrates into lactic acid. Their activity in producing lactic acid, contributing to further pH reduction, should remain unaffected by the acidic environment. In other words, the use of pH-resistant LAB can help overcome inhibition caused by the product's environment, allowing the fermentation process to proceed smoothly. This approach minimizes the effects of buffer capacity, preventing it from interfering with the fermentation of Trembesi leaves.

For the growth of lactic acid bacteria (LAB) used in the production of Trembesi leaf silage, additives are necessary. These additives serve as an energy source for LAB to survive and function during the ensiling process. The content of water-soluble carbohydrates (WSC) in the additives greatly influences the formation of lactic acid, as lactic acid-producing bacteria primarily ferment these soluble carbohydrates. Therefore, the sufficient availability of water-soluble carbohydrates is crucial for the growth of the microbes (Jamarun et al., 2014).

There are many carbohydrate sources that can be used as additives in the production of Trembesi leaf silage, but molasses contains the highest amount of soluble carbohydrates. Molasses is a by-product of the sugar processing industry and still contains a significant amount of sugar, specifically sucrose, ranging from 48-55%. Sucrose is a disaccharide composed of glucose and fructose, linked by a glycosidic bond. The chemical structure of sucrose allows water molecules to easily surround and interact with its hydroxyl (OH) groups (). These hydroxyl groups in sucrose can form hydrogen bonds with water molecules. These hydrogen bonds are essential in the dissolution process, as they help disperse sucrose molecules in water (Telleng et al., 2022).

The availability of soluble carbohydrates will be utilized by LAB for their growth. Well-growing LAB will produce high amounts of lactic acid. Increased lactic acid production will lower the environmental pH. The higher the lactic acid production, the lower the pH, which in turn helps to suppress the buffer capacity of Trembesi leaves (Yuvita et al., 2021).

In addition to the carbohydrate source used as an energy source, the fermentation duration also affects the fermentation process, ultimately influencing the quality of the silage. The conversion of sugars in the substrate into lactic acid varies depending on factors such as temperature, pH, the raw material (Trembesi leaves), and the carbohydrate source used. Carbohydrate sources with a high content of soluble carbohydrates will achieve a lower silage pH more quickly because the rapid availability of energy for LAB accelerates their lactic acid production. This lactic acid production creates an acidic environment in the silo. An acidic environment (low pH) is ideal for the ensiling process (Juwandi et al., 2019).

The faster the ensiling process occurs, the shorter the lag phase (initial growth phase) and exponential phase (growth phase) of the LAB, allowing the stationary phase to be reached more quickly. The fermentation process for silage takes 21 days, as by the 21st day the stationary phase is reached, lactic acid production has peaked, and LAB growth halts, resulting in a pH lower than 4.

MATERIALS AND METHODS

***Location and length of research*:** The research was conducted from August 2021 to January 2022 at the Animal Feed Science Laboratory, Faculty of Animal Science, Gadjah Mada University (UGM), the Nutritional Biochemistry Laboratory, Faculty of Animal Science, UGM, and the Biology and Biochemistry Laboratory, Faculty of Agriculture, Univet Bantara University.

Mass Production of L. fermentum 18b Starter: The production of silage begins with the preparation of the Lactobacillus fermentum 18b starter. The steps are as follows (Handajani, 2014). Mix three ingredients: 2000 g of corn flour, 500 g of trembesi leaf flour, and 500 g of bran. Steam the mixture for 10 minutes, then allow it to cool. b). Combine 90 g of molasses with 1400 g of cooled boiled water. c). Mix the contents of steps a and b thoroughly. d). Inoculate the mixture from step c with 10% of the LAB starter. e). Place the mixture from step d into an airtight plastic bag, press it to remove all oxygen, and then seal it. f). Incubate the sealed bag anaerobically for 3-7 days.

***Silage Production*:** Trembesi leaves are harvested and wilted for one day to achieve a moisture content of approximately 55%. The leaves are then chopped using a chopper into pieces measuring 3-5 cm. The carbohydrate sources used include bran, molasses, corn flour, fine rice flour, and cassava flour.

Next, 90 g of carbohydrate source is mixed with 1400 ml of water. After that, 3000 g of the chopped Trembesi leaves are added, along with 10% of the substrate weight (Trembesi leaves) of *Limosilactobacillus fermentum* 18b starter, which amounts to 300 g. This mixture is placed in a glass jar, compacted, and then sealed tightly. The fermentation period is conducted for 0, 7, 14, and 21 days. Silage harvesting is performed according to the respective fermentation treatments. Samples of silage are taken by placing them into plastic bags. The collected samples are divided into two parts: one part is for fresh silage to be tested for physical quality, while the other part is for drying, which will be tested for chemical quality.

***Material*:** The materials used in the research include Starter *Limosilactobacillus fermentum* 18b, Trembesi leaves, bran, cassava flour, fine rice flour, corn flour, molasses, and MRS (de Man, Rogosa, and Sharpe) agar.

***Research Parameters*:** The research parameters observed were physical quality and chemical quality. The physical qualities of silage include its pH, aroma, color, and texture. The pH of the silage is measured using a pH meter, while the aroma, color, and texture are assessed based on the criteria from the Directorate of Animal Feed (2012). Silage with excellent quality has a pleasant fruity aroma, slightly acidic, and is given a score of 25. Good quality silage has a fragrant acidic aroma and is given a score of 20. Medium quality silage has no noticeable aroma and is given a score of 10. Poor quality silage has an unpleasant odor and is given a score of 0. In terms of color, excellent quality silage has a yellowish-green hue and is given a score of 25. Medium quality silage is brownish-black and is given a score of 10. Poor quality silage is black, resembling compost, and is given a score of 0. According to the Directorate of Animal Feed (2012), silage of excellent quality has a dry texture but feels soft to the touch and is given a score of 25. Good quality silage has a slightly moist texture but does not feel wet and is given a score of 20. Poor quality silage has a wet texture and is given a score of 0. The chemical quality, which includes dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), crude fat (CFat), and ash, is measured using proximate analysis. This allows for the calculation of nitrogen-free extract (NFE) and total digestible nutrients (TDN). NFE is calculated using the following formula:

NFE (%)=100%−ash (%)−crude protein (%)−crude fat (%)−crude fiber (%)

***Experimental Design*:** This study was designed using a Completely Randomized Design (CRD) with a factorial pattern (5x4), where each treatment was repeated 6 times. The first factor is the type of carbohydrate source (broken rice, molasses, corn flour, cassava flour, and rice bran), and the second factor is the fermentation duration (0, 7, 14, and 21 days).

Statistical Analysis: The research data were analyzed using analysis of variance (ANOVA) at a 5% significance level, following a Completely Randomized Design (CRD) with a factorial pattern. If any variables showed significant differences, further analysis was conducted using Duncan's New Multiple Range Test (DMRT). All statistical calculations were performed with the assistance of the Statistical Product and Service Solution (IBM SPSS Statistics, version 26) (Afandi et al., 2021).

RESULTS

***Physical Quality*:** The measurement of the physical quality of trembesi leaf silage is presented in Tables 1, 2, 3, and 4.

Table 1. Average pH of Trembesi Leaf Silage at Different Fermentation Durations and arbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average
	0	7	14	21	
Fine rice flour	7,28±0,01	6,85±0,03	5,24±0,02	4,30±0,03	5,92±0,02
Molases	7,37±0,02	6,92±0,02	5,29±0,02	4,37±0,02	5,99±0,02
corn flour	7,97±0,01	6,89±0,02	5,31±0,02	4,38±0,02	5,96±0,01
cassava flour	7,31±0,02	6,94±0,02	5,34±0,01	4,38±0,02	5,99±0,01
Bran	7,35±0,01	6,96±0,02	5,36±0,03	4,43±0,04	6,02±0,02
Average	7,46±0,01	6,91±0,02	5,31±0,02	4,36±0,03	

Table 2. Average Smell of Trembesi Leaf Silage at Different Fermentation Durations and Carbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average
	0	7	14	21	
Fine rice flour	10,00±0,00	10,00±0,00	18,33±1,67	16,67±2,11	13,75^{s}±0,94
Molases	10,00±0,00	10,00±0,00	20,00±0,00	21,67±1,05	15,42^{s}±0,26
corn flour	10,00±0,00	10,00±0,00	13,33±3,33	16,67±3,33	12,50^{rs}±1,67
cassava flour	10,00±0,00	10,00±0,00	15,00±3,42	13,33±4,22	12,08^{r}±1,91
Bran	10,00±0,00	8,33±1,67	12,50±3,10	13,33±4,22	11,04^{r}±2,24
Average	10,00^{a}±0,00	9,67^{a}±0,33	15,83±^{b}2,30	16,33^{b}±2,99	

Note: Superscript letters that differ in the same row and column indicate a significant difference (P<0.05).

Table 3. Average Color of Trembesi Leaf Silage at Different Fermentation Durations and Carbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average
	0	7	14	21	
Fine rice flour	30,00±0,00	24,17±0,83	18,33±1,05	13,33±1,05	$21,46^{p}$±0,74
Molases	30,00±0,00	27,50±1,12	25,00±0,00	25,00±0,01	$26,88^{q}$±0,28
corn flour	30,00±0,00	23,33±1,05	18,33±1,05	13,33±1,05	$21,25^{p}$±0,79
cassava flour	30,00±0,00	24,17±0,14	19,17±0,83	14,17±0,83	$21,88^{p}$±0,45
Bran	30,00±0,00	23,33±1,05	17,50±1,12	13,33±1,05	$21,88^{p}$±0,81
Average	$30,00^{d}$±0,00	$24,5^{c}$±0,84	$19,67^{b}$±0,81	$15,83^{a}$±0,80	

Note : Superscript letters that differ in the same row and column indicate a significant difference (P<0.05).

Table 4. Average Texture of Trembesi Leaf Silage at Different Fermentation Durations and Carbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average
	0	7	14	21	
Fine rice flour	20,00±0,00	20,00±0,00	18,33±1,67	20,83±2,39	19,79±1,01
Molases	20,00±0,00	20,00±0,00	21,67±1,05	24,17±0,83	21,46±0,47
corn flour	20,00±0,00	20,00±0,00	18,33±1,67	20,00±2,24	19,58±0,98
cassava flour	20,00±0,00	20,00±0,00	18,33±1,67	20,00±2,24	19,58±0,98
Bran	20,00±0,00	20,00±0,00	18,33±1,67	19,17±2,01	19,38±0,92
Average	20,00±0,00	20,00±0,00	19,00±1,54	20,83±1,94	

Note: ns : non significant

Table 5. Average Dry Matter Content (%) of Trembesi Leaf Silage at Different Fermentation Durations and Carbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average
	0	7	14	21	
Fine rice flour	55,04±0,24	43,18±0,32	36,22±0,15	28,54±0,20	40,74±0,23
Molases	55,36±0,24	43,02±0,39	36,36±0,12	28,54±0,18	40,79±0,23
corn flour	55,24±0,35	42,98±0,38	36,23±0,12	28,54±0,18	40,74±0,26
cassava flour	55,19±0,47	42,96±0,06	36,22±0,13	28,48±0,20	40,71±0,22
Bran	55,22±0,49	42,88±0,61	36,20±0,14	28,45±0,19	40,69±0,86
Average	$55,21^{d}$±0,36	$43,00^{c}$±0,75	$36,23±0,13^{b}$	28,51±0,19a	

Note: Superscript letters that differ in the same row indicate a significant difference (P<0.05)

Table 6. Average Organic Matter Content (% DM) of Trembesi Leaf Silage at Different Fermentation Durations and Carbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average
	0	7	14	21	
Fine rice flour	51,90±0,10	52,11±0,07	52,90±0,16	53,00±0,17	52,48±0,12
Molases	52,02±0,10	52,12±0,05	52,89±0,06	53,30±0,06	52,58±0,07
corn flour	52,07±0,05	52,22±0,03	52,00±0,03	53,20±0,02	52,62±0,03
cassava flour	52,00±0,05	52,20±0,01	52,68±0,09	53,00±0,01	52,47±0,06
Bran	52,01±0,02	52,13±0,01	52,87±0,02	53,18±0,02	52,60±0,02
Average	$52,00^{a}$±0,08	$52,16^{b}$±0,04	$52,87^{c}$±0,07	$53,18^{d}$±0,05	

Note: Superscript letters that differ in the same row and column indicate a significant difference (P<0.05)

Table 7. Average Crude Fiber Content (% DM) of Trembesi Leaf Silage at Different Fermentation Durations and Carbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average
	0	7	14	21	
Fine rice flour	31,25+1,63	32,17±1,42	31.78±1,21	33,33±1,61	$32,13^{t}$±1,58
Molases	30,57±1,02	26,15±0,91	30,72±2,33	27,74±1,39	$28,79^{s}$±2,43
corn flour	26,97±0,88	26,93±1,08	20,52±0,15	20,52±0,20	$23,77^{r}$±3,31
cassava flour	21,30±3,19	20,24±,093	20,52±0,15	20,66±0,20	$18,76^{q}$±3,31
Bran	21,30±1,15	20,24±0,39	20,36±0,39	13,14±1,19	$14,03^{p}$±3,44
Average	$24,92^{c}$±6,48	$23,80^{b}$±6,60	$23,41^{b}$±7,13	$21,85^{a}$±7,93	

Note : Superscript letters that differ in the same row and column indicate a significant difference (P<0.05)

Table 8. Average Crude Fat Content (% DM) of Trembesi Leaf Silage at Different Fermentation Durations and Carbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average
	0	7	14	21	
Fine rice flour	3,20±0,64	1,41±0,09	2,89±0,19	3,00±0,12	$2,63^{q}$±0,73
Molases	2,31±1,55	3,01±0,67	1,85±0,12	2,79±0,24	$2,49^{p}$±0,48
corn flour	2,91±0,92	2,49±0,08	2,30±0,11	2,09±0,06	$2,45^{p}$±0,31
cassava flour	3,12±0,12	2,62±0,11	2,82±0,31	1,99±0,16	$2,63^{q}$±0,45
Bran	2,27±0,13	2,92±0,13	2,85±0,64	2,75±0,45	$2,70^{q}$±0,27
Average	$2,76^{b}$±0,41	$2,49^{a}$±0,58	$2,54^{a}$±0,44	$2,52^{a}$±0,43	

Note : Superscript letters that differ in the same row and column indicate a significant difference (P<0.05)

Table 9. Average Ash Content (% DM) of Trembesi Leaf Silage at Different Fermentation Durations and Carbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average
	0	7	14	21	
Fine rice flour	3,20±0,03	3,01±0,15	2,23±0,12	1,99±0,32	$2,63^{q}$±0,16
Molases	1,42±0,03	1,85±0,20	2,09±0,12	2,27±0,19	$1,91^{p}$±0,13
corn flour	2,89±0,19	2,79±0,09	3,12±0,13	2,92±0,05	$2,93^{s}$±0,11
cassava flour	3,00±0,27	2,91±0,06	2,62±0,05	2,85±0,03	$2,84^{r}$±0,10
Bran	2,31±0,16	2,49±0,03	2,82±0,13	2,75±0,02	$2,59^{q}$±0,08
Average^{ns}	2,56±0,14	2,61±0,10	2,59±0,11	2,56±0,12	

Note : Superscript letters that differ in the same column indicate a significant difference (P<0.05); ^{ns} = non significant

Table 10. Average BETN Content (%) of Trembesi Leaf Silage at Different Fermentation Durations and Carbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average
	0	7	14	21	
Fine rice flour	3,20±0,03	3,01±0,15	2,23±0,12	1,99±0,32	$2,63^{q}$±0,16
Molases	1,42±0,03	1,85±0,20	2,09±0,12	2,27±0,19	$1,91^{p}$±0,13
corn flour	2,89±0,19	2,79±0,09	3,12±0,13	2,92±0,05	$2,93^{s}$±0,11
cassava flour	3,00±0,27	2,91±0,06	2,62±0,05	2,85±0,03	$2,84^{r}$±0,10
Bran	2,31±0,16	2,49±0,03	2,82±0,13	2,75±0,02	$2,59^{q}$±0,08
Average^{ns}	2,56±0,14	2,61±0,10	2,59±0,11	2,56±0,12	

Note : Superscript letters that differ in the same row indicate a significant difference (P<0.05); ^{ns} = non significant

Table 11. Average TDN (% DM) of Trembesi Leaf Silage at Different Fermentation Durations and Carbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average
	0	7	14	21	
Fine rice flour	59,51±1,04	63,74±0,54	68,40±0,12	74,89±0,44	$66,64^{p}$±0,53
Molases	59,15±0,41	58,88±0,98	68,09±0,08	73,45±0,50	$64,89^{q}$±0,49
corn flour	60,27±0,48	62,21±0,55	67,88±0,50	74,81±0,71	$66,29^{p}$±0,56
cassava flour	57,20±0,61	63,06±0,23	68,74±0,19	74,50±0,99	$66,87^{p}$±0,51
Bran	61,82±0,41	62,92±0,61	68,76±0,16	73,77±0,34	$66,81^{p}$±0,38
Average	$59,59^{a}$±0,59	$62,16^{b}$±0,58	$68,38^{c}$±0,21	$74,28^{d}$±0,60	

Note : Superscript letters that differ in the same column indicate a significant difference (P<0.05)

Table 12. Average Lactic Acid Production of Trembesi Leaf Silage (%) at Different Fermentation Durations and Carbohydrate Sources.

Carbohydrate Sources	Fermentation Durations (Day)				Average^{ns}
	0	7	14	21	
Fine rice flour	2,26±0,02	3,55±0,002	4,35±0,02	5,47±0,11	3,91±0,038
Molases	2,27±0,02	3,54±0,002	4,37±0,02	5,48±0,11	3,91±0,038
corn flour	2,21±0,02	3,51±0,002	4,31±0,02	5,42±0,11	3,87±0,038
cassava flour	2,22±0,02	3,53±0,002	4,32±0,02	5,43±0,11	3,87±0,038
Bran	2,23±0,02	3,52±0,002	4,35±0,02	5,47±0,11	3,89±0,038
Average	$2,26^{a}$±0,02	$3,55^{b}$±0,002	$4,34^{c}$±0,02	$5,39^{d}$±0,11	

Note : Superscript letters that differ in the same row indicate a significant difference (P<0.05); ns : non signifikan

***Chemical Quality*:** The measurement of the physical quality of trembesi leaf silage is presented in Tables 5, 6, 7, and 4.

DISCUSSION

***Physical quality*:** The physical quality of Trembesi leaf silage, as reflected by its pH, color, and aroma, shows that the longer the fermentation period, the lower the pH of the silage. This indicates that extended fermentation increases lactic acid production. Higher lactic acid production leads to a decrease in the pH of the silage. Good-quality silage typically has a pH range of 3.5 to 4.5. The lowest pH values were observed in all silages with the addition of various carbohydrate sources after 21 days of fermentation. This pH reduction is influenced by the production of lactic acid.

Soluble carbohydrates are crucial in the ensiling process because they ensure the production of lactic acid. Carbohydrates undergo fermentation by microorganisms, breaking down into organic acids, mainly lactic acid, with smaller amounts of acetic and butyric acids (Telleng et al., 2022). Molasses resulted in the lowest pH at 21 days of fermentation, with a pH of 4.37, compared to other carbohydrate sources. This finding aligns with Jamarun et al. (2014), who stated that molasses contains 74.9% soluble carbohydrates, higher than broken rice at 35.53% , corn flour at 71.37%, rice bran at 69.2% (Trisnadewi & Cakra, 2009), and cassava flour at 63.29%. Soluble carbohydrates are crucial in the ensiling process because they ensure the production of lactic acid. Carbohydrates undergo fermentation by microorganisms, breaking down into organic acids, mainly lactic acid, with smaller amounts of acetic and butyric acids (Telleng et al., 2022). Molasses resulted in the lowest pH at 21 days of fermentation, with a pH of 4.37, compared to other carbohydrate sources. This finding aligns with Jamarun et al. (2014), who stated that molasses contains 74.9% soluble carbohydrates, higher than broken rice at 35.53%, corn flour at 71.37% , rice bran at 69.2% (Trisnadewi & Cakra, 2009), and cassava flour at 63.29%. The aroma produced after the fermentation process is one of the key indicators in assessing the physical quality of a sample, as it reflects whether the fermentation process has gone well. High-quality silage will have a smell similar to fermented milk due to the presence of lactic acid, rather than a pungent odor. The best silage aroma was achieved with the addition of molasses after 21 days of fermentation. This suggests that molasses, which contain a high amount of simple sugars compared to other carbohydrate sources, produces volatile fatty acids during the early stages of fermentation. The addition of *Limosilactobacillus fermentum* 18b accelerates the creation of an acidic environment. This aligns with the opinion of Raguati et al. (2022), who stated that aerobic reactions during the early stages of fermentation produce volatile fatty acids. Adding a fermentation starter accelerates the development of an acidic environment, leading to a

decrease in the silage's pH. The characteristic aroma of good-quality silage ranges from non-sour or non-putrid to slightly acidic.

The color of silage is not just an aesthetic factor but also indicates the quality and condition of the silage itself. Carbohydrate sources high in starch may produce silage with a darker color, such as brown or dark brown, due to slower and more complex fermentation processes. In contrast, carbohydrate sources rich in simple sugars ferment more rapidly, often resulting in silage with a brighter color, typically yellow or light green. The color change observed in Trembesi leaves undergoing ensiling is caused by aerobic respiration, which occurs as long as oxygen is present. Once the oxygen in the Trembesi leaves is depleted, sugars oxidize into CO_2 and water, generating heat that raises the temperature. If the temperature becomes uncontrolled, the silage may turn black, resulting in a reduction in nutritional value due to the loss of carbohydrate sources. When molasses is added as a carbohydrate source, the deterioration of silage color is relatively less drastic. During the initial phase of fermentation, lactic acid bacteria become more active, consuming sugars and producing lactic acid. At this stage, the silage color still closely resembles the original raw material, typically green or yellow. As lactic acid production peaks, the pH drops significantly, and the environment becomes more acidic. The silage then begins to take on a brighter yellow color. This is because lactic acid helps preserve the natural color of the raw material while inhibiting spoilage microorganisms. After 14 days, fermentation enters a stable phase. At this point, most of the sugars have been converted into acids, and the silage reaches a stable, low pH. The color of the silage generally remains constant beyond this phase, as long as the storage conditions remain anaerobic and free from contamination.

The texture of Trembesi leaf silage with the addition of various carbohydrate sources and different fermentation durations did not show a significant difference (**P>0.05**). The texture of silage with the addition of broken rice, corn flour, cassava flour, and rice bran declined in quality until day 14, then improved on day 21. However, with the addition of molasses, the texture improved continuously as the fermentation period increased. This may be due to spoilage in some samples when using broken rice, corn flour, cassava flour, and rice bran. In contrast, the addition of molasses allowed anaerobic fermentation to proceed perfectly, enabling *Limosilactobacillus fermentum* 18b to ferment the easily soluble carbohydrates into lactic acid. According to Raguati et al. (2022) the addition of soluble carbohydrates leads to a drop in pH, which inhibits fungal growth and results in a firm, non-slimy texture.

***Chemical quality*:** To assess the chemical quality of Trembesi leaf silage**,** a proximate analysis was conducted. The statistical analysis results showed that the addition of various carbohydrate sources, fermentation duration, and their interaction had a significant effect **(P>0.05)** on the dry matter content of the silage. The carbohydrate sources used in the production of Trembesi leaf silage provided sufficient energy for the activity of *Limosilactobacillus fermentum* 18b, allowing it to produce enough lactic acid and create an acidic environment in the silage. The decrease in dry matter content in the silage occurred due to the loss of dry matter consumed by the bacteria to sustain their activity. A 21-day fermentation period resulted in the lowest dry matter content for all carbohydrate sources. This indicates that *Limosilactobacillus fermentum* 18b was highly effective in utilizing the carbohydrate sources to produce lactic acid, which also leads to an increase in H_2O as a byproduct. Plant cells do not immediately cease activity after harvest. Respiration continues as long as carbohydrates and oxygen are available. Oxygen is required for respiration, which generates energy for cell function. During this process, carbohydrates are oxidized by plant cells in the presence of oxygen, producing CO_2, H_2O, and heat.

One parameter for the success of silage production is achieving a low pH. The low pH value is caused by the presence of microbes that produce lactic acid. The addition of different carbohydrate sources significantly affects ($P<0.05$) the lactic acid production in Trembesi leaf silage. The lactic acid content of silage is categorized as good when it ranges from 5.35% to 10.65% (Jasin, 2014). This was achieved in silage with a fermentation period of 21 days. Lactic acid is the most important indicator that signifies the fermentation process is proceeding well. Lactic acid significantly accelerates the decrease in silage pH, thus minimizing nutrient degradation caused by undesirable bacteria. Jasin (2014) states that carbohydrates serve as an energy source for microorganisms to produce lactic acid, which leads to a decrease in pH. The pH value of silage largely depends on the formation of organic acids, particularly lactic acid. The highest lactic acid content is found in silage supplemented with molasses as a carbohydrate source after 21 days of fermentation. The soluble carbohydrate content in molasses is 74.9% (Juwandi et al., 2019), indicating that the longer fermentation process, supported by the high content of soluble carbohydrates, provides more energy for microbes to produce lactic acid.

The high dry matter content in Trembesi leaf silage with the addition of carbohydrate sources can reduce dry matter loss caused by the conversion of glucose into lactic acid. Jasin (2014) stated that the increase in silage dry matter is related to the ability of inoculated accelerators to lower the pH, which inhibits the growth of ***Clostridia*** bacteria and reduces nutrient degradation. As a result, silage with added accelerators generally has higher dry matter content compared to silage without accelerators. The function of accelerators is to add dry matter, which is expected to reduce the moisture content of the silage. The reduction in dry matter content of vegetable waste silage occurs because bacteria consume dry matter to

continue their metabolic activities. Dry matter loss can occur during both the aerobic and anaerobic phases. In the aerobic phase, dry matter decreases due to ongoing respiration, where glucose (a fraction of the dry matter) is converted into CO_2**,** H_2O**,** and heat. During the anaerobic phase, glucose is transformed into ethanol and CO_2 by microorganisms. A relatively high dry matter content increases the longevity of silage, allowing it to be preserved for a longer period.

One way to assess the quality of silage is by determining its organic matter (OM) content. The average organic matter content ranges from 94.09% to 94.69%, which typically comprises the largest portion of dry matter (DM) (Rohmiyatul et al., 2019). Since OM is a component of DM, a high DM content will result in a high OM content and vice versa. This is consistent with the findings of Darma et al. (2023), who stated that OM increases as DM increases.

The high OM content in Trembesi (*Samanea saman*) leaf silage is attributed to the addition of carbohydrate sources, which also increases the levels of easily soluble carbohydrates. These carbohydrates serve as readily available energy for lactic acid bacteria to carry out their metabolic activity (Akani et al., 2025). According to Juwandi et al. (2019), the addition of accelerators or inoculants can enhance OM due to increased levels of soluble carbohydrates, which are utilized by fiber-degrading microbes such as cellulolytic bacteria. These microbes ferment carbohydrates into organic acids such as acetate, propionate, and butyrate.

The crude protein (CP) content in Trembesi leaf silage increases initially but decreases again by day 21 of fermentation. Some of the protein is broken down by protease enzymes from the plant itself and microbes during ensiling, producing amino acids and non-protein nitrogen (NPN) compounds, particularly ammonia (Darma et al., 2023). This process occurs within the first 24 to 72 hours during phases 1 and 2 of fermentation. The resulting NPN compounds are then utilized by lactic acid bacteria and other microbes for growth. According to Anwar et al. (2023), CP may increase again in later stages due to microbial growth, which synthesizes microbial proteins using nitrogen from the substrate.

Crude fiber (CF) is important for ruminant livestock. The lowest CF content in Trembesi leaf silage was observed at day 21 of fermentation. The reduction is attributed to the action of Limosilactobacillus fermentum 18b, supported by sufficient carbohydrate sources, which produce lactic acid and degrade lignocellulosic bonds (Huhtanen & Jaakkola, 1993, as cited in). This hydrolyzes the fiber fraction, including neutral detergent fiber (NDF) and hemicellulose. Juwandi et al. (2019) support this by stating that fermentation breaks down crude fiber, which improves digestibility by freeing more energy for other digestion processes.

Crude fat (CFat) content in Trembesi silage decreases after 7 days of fermentation but increases again at days 14 and 21. This initial decrease may be due to the breakdown of triglycerides into fatty acids and alcohol, some of which may evaporate (). The increase in crude fat at later stages is likely due to fatty acids produced by the active growth of Limosilactobacillus fermentum 18b, which occurs during stable fermentation (Zhao et al., 2025). The presence of carbohydrate sources supports bacterial activity and reduces fat degradation, maintaining fat levels throughout fermentation.

Ash is an indicator of mineral content in silage. The average ash content in trembesi leaf silage ranges from 4.5% to 5.79% (Akbar *et al.*, 2022). A low ash content may result from microbes utilizing available minerals. In treatments using molasses, increased ash content suggests a rise in mineral content, possibly due to the nature of the organic acids used. During ashing, these organic substances burn, increasing the ash residue (

Non-Nitrogen Extracted Material (BETN) consists of soluble carbohydrates that are quickly digested by rumen microbes. A high BETN value improves the nutritional quality of silage (Juwandi et al., 2019). The increase in BETN during fermentation is attributed to microbial degradation of structural carbohydrates such as hemicellulose, breaking them into more soluble compounds. This aligns with Huhtanen and Jaakkola (1993, as cited in), who reported that fermentation reduces CF while increasing BETN.

The TDN (Total Digestible Nutrients) value from each addition of carbohydrate sources will increase as the fermentation period lengthens. The increase in TDN is in line with the rise in the BETN (Non-Nitrogenous Extract) content of the silage. This demonstrates that fermentation enhances the breakdown of structural carbohydrates, such as hemicellulose, into more soluble materials, as indicated by the increasing BETN content. The higher the content of soluble materials, the greater the digestibility for livestock.

Conclusion**:** The best physical and chemical quality of Trembesi leaf silage, which can be used as one of the feed ingredients for livestock, is achieved by adding the bacterial additive Limosilactobacillus fermentum isolate 18b, molasses as a carbohydrate source, and a fermentation period of 21 days.

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